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Department of Homeland Security Fellowship Internship Experience at Lawrence Livermore National Laboratory

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Internship Project and Role

As a DHS intern at Lawrence Livermore National Laboratory (LLNL), I was a member of the Agricultural Domestic Demonstration and Application Program (AgDDAP) under the mentorship of Benjamin Hindson. This group is focused on developing assays for the rapid detection of animal diseases that threaten agriculture in the United States.

The introduction of a foreign animal disease to the US could potentially result in devastating economic losses. The 2001 Foot-and-Mouth Disease (FMD) outbreak in the UK cost over 20 billion dollars and resulted in the death of over 6 million animals. FMD virus is considered to be one of greatest threats to agriculture due to its high infectivity, robustness, and broad species range. Thus, export of meat and animal products from FMD endemic countries is strictly regulated. Although the disease is rarely fatal in adult animals, morbidity is close to 100%. FMD also causes overall production (i.e. milk, mass) to decrease dramatically and can reduce it permanently.

The rapid and accurate diagnosis of FMD and other foreign animal diseases is essential to prevent these diseases from spreading and becoming endemic to the country. Every hour delay in the detection of FMD is estimated to cost up to 3 million dollars. Diagnosis of FMD is often complicated by other diseases manifesting similar symptoms in the animal, such as vesicular stomatitis, bluetongue, etc. Typically, diagnosis cannot be made by clinical signs alone and samples must be sent away for testing. Depending on the test, such as in virus isolation, this can take several days.

AgDDAP had previously developed a high-throughput multiplexed polymerase chain reaction (PCR) assay for the rule-out of Foot-and-Mouth Disease and six other look-a-like diseases. This assay is intended for use in FMD surveillance, differential diagnosis in an outbreak scenario, and to establish an FMD-clean state after an outbreak. PCR based assays are favorable for multiple reasons. Viral nucleic acids can be detected in samples several days before clinical signs appear. PCR is quick with results available in a few hours. The ability to multiplex PCR allows many different diseases to be detected. Also multiple signatures can be detected for each disease, decreasing the likelihood of a false negative result due to viral mutation. The virus would have to obtain multiple mutations in the correct areas in the genome to escape detection. PCR can also be easily automated with robotics and made high-throughput, allowing close to a hundred samples to be processed at a time with minimal chance of cross over contamination.

This assay panel will be expanded in the future to screen for more diseases and also be separated into specific panels targeted at bovine, ovine, and porcine diseases. The assay is designed to be deeply multiplexed and could potentially screen for as many as 96 different diseases. The assay will also be modified to accept different types of sample matrices, including blood, milk, and tissues. This is necessary because the presence of viral nucleic acids in a particular sample matrix can be dependent on the virus, the stage of infection, and the species infected.

My part of the project was to develop protocols for the purification of viral nucleic acids from blood, milk, serum, and plasma. Previous work had identified a sufficient protocol

for the purification of viral nucleic acids from an oral swab in viral transport media (VTM.) Purification and detection of nucleic acids from complex sample matrices can be complicated by the presence of other macromolecules and inorganic compounds. Thus, we wanted to assess the performance of a commercially available nucleic acid isolation kit to purify viral nucleic acids from such sample matrices. The Ambion MagMAX-96 Viral Nucleic Acid Isolation Kit was used to purify viral nucleic acids and TaqMan reverse-transcriptase PCR (RT-PCR) was used to quantify the nucleic acid output. Purified live bovine viral diarrhea (BVD) virus was also spiked into the sample matrices and assessed via TaqMan RT-PCR.

Alien arRNA is a synthetic RNA sequence packaged in a non-infectious MS9 phage and was used to simulate live virus. The RNA sequence does not match any in GenBank, hence the name "alien." Direct spikes of alien arRNA into the PCR mix showed that TaqMan RT-PCR was sensitive down to 10 copies of alien RNA per reaction.

Alien arRNA was also spiked into serum, plasma, viral transport media (which contained an oral swab), whole blood (heparin and EDTA), and raw milk. It was found that purifying alien RNA from serum, plasma, and oral swabs placed in viral transport media (VTM) gave consistent yields. This result suggested that purification of viral nucleic acids from serum and plasma should perform similarly to oral swabs in VTM. Purification of alien RNA from whole blood and raw milk samples proved to be less reproducible due to interference from cellular DNA (i.e. mitochondrial DNA in red blood cells and genomic DNA in somatic cells of milk.) Large differences in the quantity of

purified RNA were detected. Too much cellular DNA in the sample may reduce viral nucleic acid yield. Use of a different kit that removed cellular DNA (through a DNase treatment) improved RNA recovery.

Live BVD virus (20 – 200 TCID50/mL) was spiked into VTM, serum, whole blood, and raw milk and nucleic acids were purified and detected with TaqMan RT-PCR. Previous work has shown that this concentration range falls around the threshold median fluorescence intensity (MFI) in multiplex PCR. An MFI above this threshold value is indicative of a sample positive for BVD. TaqMan RT-PCR was sensitive down to 20 $TCID_{50}$/mL in VTM, though sensitivity varied in serum, whole blood, and raw milk. Purification of BVD RNA from serum, whole blood, and milk did not produce a consistent yield. This was expected of whole blood and milk due to the presence of cellular DNA. However, inconsistency was not expected from serum since its performance with alien RNA was similar to oral swab in VTM. This inconsistency may be due to degradation of the viral stock. Since the viral stock was not re-titred prior to the experiment, it cannot be determined if the virus is still intact or at the correct titre.

Protocols for purification of viral nucleic acid from whole blood and raw milk need to be further optimized to improve reproducibility. More work on nucleic acid purifications with spiked live virus need to be completed. However, based on the alien RNA purifications from serum and plasma, live virus nucleic acid purifications from these sample matrices should perform well. Future work will also include purification of

nucleic acids from other live viruses in the same sample matrices and detection via multiplex PCR.

The results from this project were partially presented at the LLNL Summer Student Poster Symposium on August 10, 2006. These results will also be published in a report submitted to the USDA and DHS.

Internship Impact on Career Planning

Interning at LLNL has given me the opportunity to experience research in a government setting. I have been in academic research for the past five years and have had limited experience in the government arena. I have thought about pursuing a career in government research and I have enjoyed the chance to work at LLNL. It has been extremely exciting to see the talents of many people from different disciplines come together and create not only new ideas but carry them through to actual products. One of my interests has been in developing diagnostics for the detection of animal and/or human disease. I was lucky to be able to work on a project doing just that.

I was extremely fortunate to be able to attend a demonstration of the high-throughput capabilities of the AgDDAP multiplexed PCR assay at the Diagnostic Laboratory of Colorado State University. It is hoped that this assay will eventually become a standard procedure for animal disease testing at diagnostic laboratories around the nation. AgDDAP members, staff from DHS, USDA, and diagnostic laboratories, and industry members gathered in Fort Collins to observe the capabilities of the assay and share ideas

of where this technology may go. This was the first time I was actually able to observe the assay being carried out from nucleic acid isolation through detection using the robotics. I was also able to see how many different people with different expertise, ranging from molecular biology to computer science, had to work together to develop this product. I also had the opportunity to see how people on the receiving end of the product reacted and what questions and concerns they had.

The DHS seminars and tours of LLNL facilities gave an overview of other projects occurring at LLNL. It is very easy in such a large facility and in science to get too focused in your specific area. Having these seminars and tours allowed me to step back and take a look at the bigger picture of what people are doing to defend the US.

The different lectures I attended were mostly from non-LLNL scientists. It was interesting to see how collaborations could be formed between government and academia. It was also a good reminder that basic science research is very important to continue to feed the development of technologies and products.

LLNL did an excellent job bringing together all of the summer interns. All of the summer interns, regardless of the program they were affiliated with, were invited to various lectures, workshops, and socials. It was a great way to meet people from various fields and at different stages of their education. Students early in their education, some as early as high school, benefited from the ability to interact with older students. Working in a lab at LLNL, in itself, is very conducive to mixing diverse people since many of the labs are

shared between multiple groups. I enjoyed being able to interact with post-doctoral students and other graduate students to develop ideas for where I might go and what I might do after graduate school. I also enjoyed being around the undergraduates and serving as a resource for graduate school life.

Areas of research to accomplish DHS mission and goals

It is important that basic research at the university and government level is continually funded. It is through basic research that novel ideas can be developed and used as the basis for new technology. New technologies enable us to defend our borders, prevent acts of terrorism and, if need be, respond to them. Funding basic research at the university levels also provides the support needed to train tomorrow's leaders. As a graduate student, I am very appreciative of the funding from DHS to continue my education. I hope that I can continue to support the DHS mission in my future career, whatever it may be.

Lectures and Activities Attended

AgDDAP meetings and events:

June 28, 2006: Lab meeting

July 7, 2006: Sample collection, B&G Dairy, Davis, CA

July 27, 2006: AgDDAP High-throughput Demonstration, Diagnostic Laboratory, Colorado State University Veterinary Teaching Hospital, Fort Collins, CO

DHS Seminar series:

June 20, 2006: Tony Farmer: Terrorism and Terrorism Network

July 18, 2006: Reg Beer: Microdroplet PCR

August 1, 2006: Kevin Ness: Microfluidic technologies to purify, concentrate and separate biological samples

August 8, 2006: Ellen Raber: Restoration and Decontamination

Symposiums

August 10, 2006: LLNL Student Poster Session; Presented poster entitled:

Nucleic Acid Purification from Clinical Sample Matrices for Foot-and-Mouth Disease Diagnostics

Lectures and Panels

June 22, 2006: Panel – Hot Topics in Science: What's next?

Moderator: Eveline Dube, Division Leader, CAR, Computation

Elbert Branscomb , Associate Director, Biosciences

Anantha Krishnan, Director, Micro/Nano Technology Center, Engineering

Edward Moses, Associate Director, NIF

Mark Musculus, Combustion Research Facility, SNL

June 29, 2006: Seminar: Evaluation of Nondestructive Technologies

July 21, 2006: Dr. Joseph DeRisi, Viral Discovery Chip, University of California, San Francisco

August 14, 2006: Biosecurity lecture: Dr. Robert Nordgren- Use of Recombinant Vaccines in Animal Health

August 17, 2006: Biosciences Seminar: Dr. David J. Brenner, Columbia University: Minimally-Invasive, Very High Throughput Radiation Biosimetry after a Radiological Event

August 21, 2006: Biosciences Seminar: Dr. Michael Mahan, UC Santa Barbara – Cross-Protective Vaccines for Emerging Infectious Diseases

Tours

June 29, 2006: LLNL Library Tour and Orientation

July 11, 2006: National Ignition Facility, LLNL

August 2, 2006: Joint Genome Institute

Networking

June 24, 2006: LLNL River Rafting Trip

June 29, 2006: Student Welcome Reception